To Check the Efficacy of *Urtica dioica* andRoman chamomile (*Chamaemelum nobile*) Against Multi-drug Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* Isolated from Post-operative Surgical-site Infections

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INTRODUCTION

Prior to Joseph Lister's introduction of the antisepsis principles in the late 1860s, postoperative wound infections will be a significant contributor to sepsis and fatalities. An everyday healthcare issue is post-operative wound infection. A number of biological pathways interact at the molecular level during the intricate process of wound infection. High morbidity and mortality are caused by wound infections [1].

Post-operative infections poses a significant challenge for patients undergoing surgical procedures andamong the most widely reported healthcare-associated illnesses in the world.Post-operative infections make up more than 30% of infections acquired in a medical setting[2].Endogenous infections, which develop from bacteria already existing in the patient's body, are the primary cause of infections [3, 4].The fact that various human skin locations exhibit varied microbiota compositions is widely established [5, 6].However, external factors like unclean operating rooms and contaminated surgical instruments could potentially be the cause of the causative agents. Unsurprisingly, bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, coagulase-negative *staphylococci*, and Enterococcus species will make up the majority of the isolated causal agents (either exogenous or endogenous pathogens) [7, 8].

Escherichia coli and *Acinetobacter baumanni* are indeed well-known multi-drug resistant Gram's negative bacteria that secreteextended-spectrum beta-lactamases (ESBLs) has been a significant concern in the South-East Asian region, as well as in other parts of the world[9].Gram-positive With the development of methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant enterococci, both Enterococcus species and *Staphylococcus aureus* have demonstrated growing resistance to last-line antibiotic therapies [10].

Postoperative infections are always associated with higher severity, as would be predicted, with longer use of invasive medical devices and prolonged hospital stays in hospital wards, and ICUs[11].Moreover, the extended usage and administration of antibiotics with insufficient dose has resulted in drug resistance [12, 13, 14].The issue of inappropriate antibiotic usage, which is

widespread in healthcare institutions, has been uncovered by extensive study conducted in some countries [15, 16, 17].

Morbidity and death among hospital inpatients are frequently brought on by surgical site infections (SSI). They are largely responsible for nosocomial infections among hospital inpatients. As a result, they can serve as stand-in indicators for nosocomial infections. The most frequent nosocomial infection among surgical patients, SSIs have also been found to be the main culprit behind unfavorable outcomes from operations[18, 19].

Surgical site infections, a leading contributor to nosocomial infections worldwide, account for 20-25% of all hospital acquired infections(20, 21).Surgical site infections are known to cause high rates of mortality, and morbidity which are estimated to range from 2.5% - 41.9% globally [22, 23].

Traditional medical practitioners from all over the world have treated a variety of ailments with medicinal herbs. In order to provide for their basic healthcare, almost 60% of the world's population still uses medicinal herbs.

Urtica dioica (stinging nettle) is annual and perennial herb in the family *Urticaceae*. This plant is widespread throughout South Asia and the Indian subcontinent, and its usage as medicine extends back a long time.Finding new antimicrobial compounds is made easier and more effective by using medicinal plants. They include active ingredients that have a variety of medical applications. It has been suggested that the plant extracts be used as antibacterial agents.

Roman Chamomile, scientifically known as *Chamaemelum nobile*, is perennial herb that belongs to the daisyfamily,*Asteraceae*. It is native to Europe and is also commonly known as English chamomile, garden chamomile, or true chamomile. The plant has small, daisy-like flowers that have white petals and yellow centers. The leaves of the plant are feathery and aromatic, and the plant has a pleasant, apple-like fragrance. Roman chamomile is often used in teas, essential oils, and skin care products due to its calming and soothing properties.

REVIEW OF LITERATURE

When a skin incision is made during a surgical procedure, there is a risk of developing an infection in the surgical wound. This type of infection is known as a surgical site infection (SSI).Most surgical wound infections develop 30 days after surgery. A surgical wound that is infected may be red, uncomfortable, or hot to the touch, and it may even be flowing pus. You can feel ill and have a fever.

Drug-resistant microorganisms have endangered the efficacy of currently available medications and continue to be the main factor in treatment failure. The burden of illness and death has moved to emerging countries as the frequency of risk factors associated with economic transition has increased. Once believed to be miracle treatments, antibiotics can no longer treat resistant germs. Many bacteria that are extremely hazardous and multi-drug resistant have been found. Since the number of freshly approved antimicrobial drugs has drastically decreased in recent years, it is anticipated that the supply of powerful antimicrobials would soon run out [24].

Staphylococcus aureus is the bacteria that is most frequently cultivated from SSIs. When a viscus is opened, its tissues, including the big bowel, are likely to be infected by a number of germs. For instance, SSI following colorectal surgery might be brought on by enterobacteriaceae and anaerobes. The presence of a foreign body, such as a prosthetic implant or medical device, during surgery can enhances the chance of Surgical Site Infection. In such an environment, non-pathogenic microorganisms like *Staphylococcus epidermidis* may also result in SSI [25, 26].

The studies conducted have investigated multi-drug resistant organisms (MDRO) infections in various surgical populations. These studies have explored the prevalence and impact of MDRO infections in patients undergoing different types of surgeries, including transplant, oncologic, urgent, general, and gynecological surgeries [27, 28].

Gram-positive species, such as Coagulase-negative bacteria, commonly cause nosocomial blood stream infections. E. coli and other Enterobacteriaceae family members, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* are among the bacteria that almost always make up true bacteremia (Gram-negative) [29, 30].

According to bacteriological investigations, SSIs are a worldwide problem, and the etiological agents that cause them can differ depending on the patient, the surgeon, the treatment, the hospital, or even the ward. Gram-negative bacteria are indeed becoming more common and are a significant source of potentially fatal infections in many hospitals and healthcare settings. The situation has gotten worse due to antimicrobial resistance (AMR), which is brought about by the improper use of broad-spectrum antibiotics. Poor infection control procedures, congested hospitals, and incorrect antimicrobial use all contribute to the problem in developing countries [31].

Microbial contamination of the surgical site, which may be induced by exogenous or endogenous, is the first stage in the development of surgical site infections (SSIs).

The body of an individual has a variety of microbes called as the endogenous flora or microbiota. These microorganisms can be found in various sites of the body, including the skin, mucosal membranes, and hollow viscera (such as the gastrointestinal and respiratory tracts). The most prevalent endogenous pathogens are Coagulase-negative *Staphylococcus aureus, Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and Staphylococci are more frequent in abdominal surgery than Gram's negative bacilli and anaerobic microbes. Exogenous flora from the theater room, such as the air, equipment, supplies, and crew, may be present. One of the biggest risk factors is the quantity and pathogenicity of the germs. More frequently, infections from post-operative wounds are being caused by high pathogenicity organisms like MRSA. Perhaps as a result of the widespread use of broad-spectrum antibiotics.

SSIs continue to be a problem for postoperative patients, despite the use of prophylactic antibiotics both before and after surgery, as well as other preventive measures such as improved sterilization processes, surgical skill, operating room ventilation, and barrier use. The main cause of this has been identified as the emergence of antimicrobial resistance brought on by excessive antibiotic use. Use of antibiotics improperly raises the pressure of selection, which promotes the growth of pathogenic microorganisms with drug resistance [32].

Urtica dioica has a number of pharmacological properties, including antioxidant, antibacterial, anticancer, analgesic,anti-inflammatory, and antiviral properties. Many valuable chemical

compounds, such as saponins, flavonoids, phytosterols, amino acids,proteins, tannins and will be discovered in phytochemical studies.

Chamaemelum nobile has a wide range of pharmacological activities, including antibacterial, antiulcer, and anti-inflammatory properties. Terpenoids, flavonoids, coumarins, and other components such as tiglic acid esters, and angelic, fatty acid, anthemic acid, and choline are active components of Roman chamomile.

RESEARCH GAP

1. Limited research on herbal remedies: There is a lack of comprehensive scientific studies investigating the potential antimicrobial properties of *Urtica dioica* and *Chamaemelum nobile* specifically against multi-drug resistant strains of *Pseudomonas* aeruginosa and *Staphylococcus aureus*. Although these herbs have been traditionally used for medicinal purposes, there is need for robust scientific evidence to support their efficacy in combating drug resistant infections.
2. Specificity to post-operative surgical site infection: While there is existing research on the antimicrobial properties of various plant extracts, there is lack of studies specifically targeting post-operative surgical site infections caused by multi-drug resistant *P.aeruginosa* and *S.aureus*. It is crucial to investigate the effectiveness of *Urtica dioica* and *Chamaemelum nobile* in this specific context, as surgical site infections are a significant concern in healthcare settings.
3. Comparative studies with conventional antibiotics: To establish the potential of *Urtica dioica* and *Chamaemelum nobile* as alternative or adjunctive treatments, it is essential to compare their efficacy with conventional antibiotics commonly used to treat multi-drug resistant *P.aeruginosa* and *S.aureus* infections. Comparative studies will help determine whether these herbal remedies can provide comparable or standard pharmaceutical treatments.
4. Mechanism of action and active compounds: Understanding the underlying mechanisms of action and identifying the active compounds responsible for the antimicrobial activity of *Urtica dioica* and *Chamaemelum nobile* is essential. Further research should focus on elucidating the specific bioactive compounds present in these herbs that contribute to their

antimicrobial properties. This knowledge will aid in the development of standardized herbal formulations or targeted treatments.

5. Clinical studies: While initial laboratory studies may indicate the potential efficacy of *Urtica dioica* and *Chamaemelum nobile* against multi-drug resistant *P.aeruginosa* and *S.aureus*, it is crucial to validate these findings through clinical studies. Further investigation is needed to assess the safety, dosage, and effectiveness of these herbal remedies in real-world scenarios, such as in animal models or clinical trials involving post-operative patients.

Addressing these research gaps will contribute to the scientific understanding of *Urtica dioica* and *Chamaemelum nobile* efficacy in treating multi-drug resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections, particularly in the context of post-operative surgical site infections. The findings could potentially lead to the development of novel therapeutic strategies or complementary treatments for these challenging infections.

AIMS AND OBJECTIVES

1. To collect the samples from:
a. Postoperative patients and
b. Medicinal herbs.
2. To isolate the *Pseudomonas aeruginosa* and *Staphylococcus aureus* from post-operative surgical site infection.
3. To select the multi-drug resistant strains of *Pseudomonas aeruginosa&Staphylococcus aureus* using the Kirby-Bauer disc diffusion method.
4. To identify the isolated bacterial strains from:
a. Morphological
b. Biochemical and
c. Molecular (by 16S rRNA sequencing) method
5. To determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts against isolated multi-drug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* using the agar well diffusion method.

SIGNIFICANCE OF WORK

An infection that develops in the wound from an invasive surgical operation is called a surgical site infection. Leading to extended hospital stays, higher hospital expenses, higher rates of reoperation, and even higher mortality rates, SSI is a major contributor to hospital morbidity. Even though the majority of infections can be treated with medicines, SSIs are still a significant source of morbidity and mortality following surgery.SSI is the most common reason for readmission to the hospital following surgery and can result in mortality in approximately 3% of patients.

Urtica dioica and Roman chamomile are medicinal plants that have been traditionally used for their anti-inflammatory properties. It is possible to find novel antimicrobial drugs that can be utilized to treat these infections by evaluating their effectiveness against multi-drug resistant strains of *P.aeruginosa* and *S.aureus*.

PROPOSED WORK PLAN

1. Isolation of multi-drug resistant *Pseudomonas aeruginosa*, and *Staphylococcus aureus* from postoperative patients admitted to different hospitals (IIMT Life Line Hospital, LLRM Medical College Hospital, and Nutema Hospital, Meerut, U.P).
2. Identification of the isolated multi-drug resistant bacteria using morphological, biochemical, and molecular (16S rRNA Sequencing) methods.
3. To check the efficacy of *Urtica dioica*(Stinging nettle) and *Chamaemelum nobile*(Roman chamomile) against isolated multi-drug resistant *Pseudomonas aeruginosa* and*Staphylococcus aureus* using the Agar-well-diffusion method.
4. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of a plant extract against isolated pathogens.
5. Statistical analysis of the data.
6. Ethical consideration of the patients.

MATERIAL AND METHODS

1. SAMPLE COLLECTION

a. Post operative surgical site infection patient sample collection: Each patient suspected of having SSI will have a sterile pus swab or wound swab and a fluid aspirate collected aseptically. Each sample will be labeled with the date of sample collection, the method of collection, and the patient's information. Samples will be collected from (IIMT Life Line Hospital, LLRM Medical College Hospital, and Nutema Hospital, Meerut, U.P).

b. Medicinal herbs sample Collection: The collection of *Urtica dioica* (fresh leaves) and Roman chamomile (fresh flowers) from the Himalayan region.

2. ISOLATION OF BACTERIAL STRAINS

The collected samples will be cultured on different culture media (Nutrient Agar, Cetrimide Agar, MacConkey Agar, Mannitol Salt Agar, and Blood Agar) and will be incubated at 37℃ for 24 hours by dilution streaking into 4-quadrants.

3. IDENTIFICATION OF BACTERIAL ISOLATES

a. Morphological: shape, size, color, and arrangement.

b. Biochemical: catalase, coagulase, oxidase, mannitol, lactose, urease, indole, H_2S, NO_3, DNase, citrate, and tellurite test.

c. Molecular (by 16s rRNA sequencing): Amplification and sequence analysis of the 16S rRNA gene are commonly used techniques for detecting and identifying bacteria in clinical samples.

4. ANTIBIOTIC SUSCEPTIBILITY TEST(Kirby-Bauer Disc diffusion method; 1950)

Antibiotic susceptibility testing will be performed on the isolated pathogens using the Kirby-Bauer disc diffusion method on nutrient agar or Mueller-Hinton agar.The diameter of the zone of inhibition will be measured in millimeter and will be interpreted in accordance with CLSI guidelines.

5. PREPARATION OF THE PLANT EXTRACTS(Solvent extraction)

Preparing an ethanolic extract involves the extraction of bioactive compounds from plant material using ethanol as the solvent. Steps for preparing an ethanolic extract:

Select the plant material: Choose the plant material that contains the bioactive compounds that want to extract. It is important to use fresh and healthy plant material for better extraction.

Grind the plant material: Grind the plant material into a fine powder using a blender. This increases the surface area of the plant material and facilitates the extraction process.

Soak the plant material in ethanol: Place the plant material in a container and cover it with ethanol. The ethanol should be of high purity; typically, 70% ethanol is used. The ratio of plant material to ethanol varies depending on the type of plant material and the desired concentration of the extract. Typically, a ratio of 1:10 (Plant material: Ethanol).

Extraction: Allow the plant material to soak in the ethanol for a period of time (72 hours). The container should be kept in a dark and cool place to prevent degradation of the bioactive compounds.

Filtration: After extraction, filter the extract to remove any solid particles using filter paper or a fine mesh strainer.

Concentration: the extract can be concentrated by evaporating the ethanol using a rotator evaporator or by using a freeze dryer. The concentration of the extract can be adjusted by evaporating off more or less of the ethanol.

After the solvent has been completely evaporated, the extracts will be dissolved in Dimethyl Sulfoxide (DMSO) to a final concentration suitable for further experimentation or testing.

Storage: Store the extract in a dark and cool place in an airtight container to prevent oxidation and degradation. The extract can be stored for several months or longer depending on the stability of the bioactive compounds.

6. ANTIMICROBIAL ACTIVITY OF THE EXTRACTS AGAINST PATHOGENS

It will be done using Pseudomonas aeruginosa and Staphylococcus aureus that have been isolated from post-operative SSIs. We'll use the Agar Well Diffusion technique (M.W. Beijerinck in 1889) to assess the antimicrobial activity of the extracts. The 100 μl standardized inoculum (108 CFU/ml) of each bacteria will be added to Muller-Hinton Agar (MHA) plates before being dispersed with sterile swabs. Each agar plate containing the bacterial inoculum will have a well of 6 mm in diameter created with a sterile borer. The well bottoms will be sealed with a little molten agar medium. Each plant's extract was put into a well in a volume of 25 μl (25 mg/well). A control well will be created in each plate by adding 25 μl of newly made sterile water. To allow the plant extract to diffuse into the agar, the plates will be left at room temperature for 15 minutes. Each well's inhibitory zone will be measured in millimeters following a 24-hour incubation period at 37°C.

7. DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) (Alexander Fleming in 1980)

The minimum inhibitory concentration (MIC) of the extracts will be calculated using the lowest concentration of the bacterial species. Macro-broth tube dilution test will be applied for this purpose. In this test, a twofold dilution of the extract will be made in tubes with 1 ml of MHB and a bacterial suspension with a turbidity of 0.5 McFarland. At 37 °C, these tubes will be incubated for 24 hours. The first tube in the aforementioned sequence that shows no evidence of observable development will be regarded as the MIC. Three times will this process be carried out.

8. DETERMINATION OF THE MINIMUM BACTERICIDAL CONCENTRATION (MBC) (Alexander Fleming in 1980)

A loop full of bacteria from test tubes that failed to show any apparent growth during the MIC assay will be grown on MHA and will be incubated at 37°C for 18 to 24 hours to determine the MBC (minimum bactericidal concentration). MBC will be given to the highest dilution that did not result in colony formation on MHA.

9. STATISTICAL ANALYSIS

The Statistical Package for Social Science (SPSS) programme will be used to analyse the data.Test significance will be done using Chi-square test. A p-value of less than 0.05 indicates statistical significance.

10. A CONSIDERATION OF ETHICS

The study will be examined by the ethical committee of the Hospital as well as the IIMT University for the ethical clearence certificate. Patients, or parents in the case of children, will be informed of the study's goals, and those who provided their informed consent will be included in the investigation.

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